International Journal of Agricultural
Science and Research (IJASR)
ISSN(P): 2250-0057; ISSN(E): 2321-0087
Vol. 4, Issue 3, Jun 2014, 175-182

MICROPROPAGATION OF *COCHLOSPERMUM RELIGIOSUM* (L.) ALSTON THROUGH NODAL EXPLANT

A. SASIKALA[1], M. LINGA RAO[2] & N. SAVITHRAMMA[3]

[1,2]Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India

[3]Professor, Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India

ABSTRACT

Cochlospermum religiosum (L) Alston is an important medicinal plant belonging to the family Cochlospermaceae. The gum, stem bark and root are traditionally used to cure different diseases. The present study aimed to develop the multiple shoots from *Cochlospermum religiosum* through nodal explants. Shoot induction was achieved on Murashiage and Skoog (MS) medium fortified with 2 $mg1^{-1}$ BAP, 2 $mg1^{-1}$ KN and 2 $mg1^{-1}$ BAP with 0.1 $mg1^{-1}$ IAA. The shoots were sub cultured after two weeks on different concentration of BAP for multiple shoot induction. After four weeks the shoots were excised and sub cultured for rooting on half strength MS medium fortified with BAP 0.5 $mg1^{-1}$ and IAA 2.0 $mg1^{-1}$. The *in vitro* generated plantlets were successfully acclimatized in pots containing vermiculite and soil. The survival rate is 50 to 65 percent.

KEYWORDS: *Cochlospermum religiosum*, Nodal Explants, Micropropagation

Abbreviations

BAP-Benzyl adenine (6-benzyl aminopurine), HCl - Hydrochloric acid, $HgCl_2$-Mercuric chloride, IAA- Indole-3-acetic acid, IBA-Indole-3-butyric acid, LAF - Laminar air flow, mg - Milligram (s), NAA- 1-naphthalene acetic acid, NaOH-Sodium Hydroxide, PGR-Plant Growth Regulator, pH-Negative logarithm of hydrogen ion concentration

INTRODUCTION

Cochlospermum religiosum (L) Alston is a sparsely branched small tree, belonging to the family Cochlospermaceae. It is commonly called as Yellow Silk Cotton, Buttercup Tree and Torchwood Tree because of flowers are large, bright golden yellow and seeds covered with silky hairs. The powder of *C. religiosum* stem bark and root is traditionally used for fertility and ash of fruit mixed with coconut is used for the treatment of scabies (Goud *et al*., 2005). The gum of *C. religiosum* is also found to be an ingredient of unani medicine. Qurs-e-Sartaan Kafoori which is used for Styptic, Antipyretic, Phthisis, Tuberculosis, Hectic fever and Qurs-e-Suzak Cicatrizant, Diuretic and Gonorrhea. These formulations were found to possess good antibacterial and antifungal activity (Cecilie *et al*., 2005). Hence the plant has been studied for antimicrobial activity (Sasikala and Savithramma, 2012); quantitative and quantitative of phytochemicals (Sasikala and Savithramma, 2012; Sasikala *et al*., 2013), Histochemical (Sasikala *et al*., 2013) and *in vitro* propagation through shoot tip culture (Sasikala and Savithramma, 2012). The plant is propagated by seeds and also multiplied by budding, cuttings or air layers. Severe anthropogenic pressures, habitat destruction and low seed germination scarces natural regeneration in the wild. The application of a reliable *in vitro* clonal propagation system would provide an

alternative method of propagation to meet the pharmaceutical needs and for effective conservation of plant species. *In vitro* propagation of plants holds tremendous potential for the production of high quality plant based medicines (Murch *et al*., 2000). This protocol can assure that a stable supply of this medicinally important plant irrespective of any seasons and may serve as a better source for biological active compounds.

MATERIALS AND METHODS

The fruits were collected from the mature plant on the Ghats of Tirumala hills. The silky hairs were removed from the seeds. The seeds were thoroughly washed in running tap water for about 15 minutes to remove surface dust particles and were subsequently rinsed with liquid detergent (teepol – 1%) for 5 minutes and then rinsed with distilled water to wash off the detergent. They were then disinfected with 0.1% $HgCl_2$ solution for 2-3 minutes followed by repeated rinsing with sterile distilled water.

Full strength and half strength media were prepared with various concentrations of sucrose. The medium was gelled with 1% agar and pH was adjusted to 5.6 to 5.8 using 0.1 N NaOH or 0.1N Hcl solution before autoclaving. 25 ml of medium was taken in each culture vessel and capped tightly. Then the cultures were autoclaved at 121^0 C for 15 minutes. The surface sterilized seeds were inoculated under aseptic conditions in LAF chamber. The cultures were incubated at 25 ± 2^0 C under fluorescent light. The aseptic nodes were excised and inoculated on to the media with different concentrations of BAP and Kinetin separately ranging from 0.5mg l^{-1} to 2.5mg $l^{-1.}$ The cultures was incubated at 25 ± 2^0 C under fluorescent light. After shoot initiation they were sub cultured for multiple shoot induction with 2 mg l^{-1} BAP. The shoots were then transferred to rooting media with different concentrations of BAP, IAA and IBA supplemented with charcoal. The rooted plantlets were introduced in to plastic bags with vermiculite, soil mix for acclimatization. In all experiments 20 replicates were used and each experiment was repeated thrice. All the results were statistically analyzed.

RESULTS AND DISCUSSIONS

In the present study the seeds of *Cochlospermum religiosum* inoculated on basal MS medium with 0 to 4% Sucrose. Seeds showed germination after two weeks (Figure 1a). 100% of seed germination was observed in MS medium without sucrose, whereas higher percentage of seed germination with less concentration of sucrose was observed. The seeds were failed to germinate on MS medium with 3% and 4% sucrose concentrations (Table-1). This is due to the presence of heteropolysaccharides in the plant parts (Ojha *et al*., 2008). After 5 weeks the seedlings attained the size of 6 to 7 cm. Nodes were excised from aseptic seedlings and inoculated on MS medium with different concentrations of BAP, KN, IAA and NAA to test the shoot regeneration capacity. 2 mg l^{-1} BAP was proved to be more effective when compared with other concentrations and other plant growth regulators. Cytokinins especially BAP, release lateral buds from dormancy and initiate shoot formation. 2 mg l^{-1} BAP in the nodal explants induced 80% shoot regeneration (Figure 1b), highest mean number of shoots i.e 3.9 and increase in shoot length i.e 4.2. 40% shoot regeneration, 2.0 mean number of shoots and 3.5 increases in shoot length were observed in concentration of 2 mg l^{-1} KN and the combination of 2 mg l^{-1} BAP with 2 mg l^{-1} KN, 40% shoot regeneration was observed (Table -2).

Exogenously supplied growth regulators regulate the callus induction. Callus induction was usually associated with high auxin to low cytokinin ratio (Centeno *et al*., 1996). Nodal explants of *Cochlospermum religiosum* such as *in vitro* raised, nodal segments were inoculated for callus induction with various concentrations of auxins and cytokinins in MS

medium. Explants tested for callus formation, Combination of BAP with NAA in MS medium was found to be best in inducing more amount of callus. Green colored compact highly morphogenic calli was produced from nodal explants on MS medium supplemented with 1.0 mg l^{-1} BAP in combination with 0.5 mg l^{-1} NAA (Figure 1c). NAA was proved to be more effective in callus induction followed by IAA and IBA (Table-3). The production of multiple shoots from nodal explants through *in vitro* propagation was caused by stimulating precocious axillary shoots to overcome the dominance of shoot apical meristems (Savithramma *et al*., 2011). Phytohormones were the crucial factors affecting regeneration of shoots from nodal explants. Cellular differentiation and organogenesis in tissue and organ culture have been found to be controlled by an interaction between different phytohormone concentrations (Savithramma *et al*., 2011). Multiple shoot regeneration 2.5 mg l^{-1} BAP produced maximum percentage of response (67%) with mean number of 4 multiple shoots from Nodal derived callus. KN was less effective with 45% of response and 2.5 numbers of multiple shoots at 2 mg l^{-1} concentration (Table-4; Figure 1d).

The *in vitro* obtained shoots were excised and transferred to half strength M.S medium supplemented with charcoal and combination of IAA and BAP at different concentrations. At 2 mgl^{-1} IAA and 0.5 mg l^{-1}BAP profused rooting was observed with a bulging at the base of stem. A maximum of 25 roots were developed at this concentration (Figure 1e). Second highest response was observed at 0.2 mg l^{-1}BAP in combination with 1 mg l^{-1} IAA (Table-5).

The effectiveness of the combination of cytokinins and auxins in producing roots was observed in Sugarcane genotypes BO91, BO110, BO120, BO128 (Ranju *et al*. 2004), but in many cases auxin alone can induce rooting. In Indian banana cv Rajeli, NAA alone induced roots (Kulkarni *et al*., 2006). In *Bixa orellana* L. (Rammurthy *et al*. 1999), *Albizzia amara* (Rammurthy and Savithramma 2003), *Cassia alata* (Rammurthy and Savithramma 2002), *Talinum cuneifolium* (Savithramma *et al*. 2010) and *Poncirus trifoliate* (Ashuthosh *et al*. 2009) either IBA or NAA alone induced rooting, whereas in *Gmelina arborea* (Amod *et al*. 2010) the combination of IBA and NAA was proved effective in producing roots.

CONCLUSIONS

In the present study 2 mg l^{-1} IAA in combination with 0.5 mg l^{-1} BAP is effective in causing root induction and profused rooting, whereas 4.9 µM IBA produced optimum number of roots in same plant when nodal segments used as explant. The roots are light brown in colour, linear and solid. *In vitro* regenerated plants with profuse roots were removed from culture vessels and washed carefully to remove adhered agar and nutrients. They were transferred to plastic bags containing vermiculite and soil (1:3) and maintained for two weeks in a growth chamber for their acclimatization. About 60% of the plantlets survived and are growing well under field conditions (Figure 1f).

The present study resulted in a reliable and effective protocol for *in vitro* regeneration of the important medicinal tree taxa *Cochlospermun religiosum* to meet the increasing demand of the herbal medicines instead of chemical drugs.

REFERENCES

1. Amod K, Sarfaraj A and Naseem Md (2010). *In vitro* plant regeneration from Organ cultures of *Gmelina arborea* Roxb. *J. Indian bot. soc*. 89:197-203.
2. Ashuthosh, AM, Sathyavathi S and Singh SK (2009). Micro propagation of Trifoliate Orange (*Poncirus trifoliate*). *Indian J. Plant Physiol*. 14: 39-43.

3. Cecilie SN, Drissa D, Kari I, Terje EM, Tsukasa M and Hiroaki K. Medicinal use of *Cochlospermum tinctorium* in Mali Anti-ulcer, radical scavenging and immunomodulating activities of polymers in the aqueous extract of the roots. *J Ethnopharmacol* 2005; 255-269.

4. Centeno ML, Rodriquez A, Feito I and Fernandez B. Relationship between endogenous auxin and cytokinin levels and morphogenic responses in *Actinidia deliciosa* tissue cultures. *Plant Cell Rep* **1996;** 16: 58-62.

5. Goud PS, Murthy KS, Pullaiah T and Babu GV. Screening for antibacterial and antifungal activity of some medicinal plants of Nallamalais-Andhra Pradesh, *India. J Econo Taxono* Bot 2005; 29: 704-08.

6. Ignacimuthu. S. (1997). Plant Biotechnoloyg, Oxford and IBH Publishing Co. Pvt. Ltd., p.180.

7. Kulkarni VM, Suprasanna P and Bapat VA (2006). Plant regeneration through multiple shoot Formation and somatic embryogenesis in a commercially important and endangered Indian banana cv. Rajeli. *Curr. Sci*. 90: 842-846.

8. Murch, S.J., Krishna Raj, S. And Saxena, P.K. (2000). Tryptophan is a precursor for melatonin and serotonin biosynthesis in *In vitro* regenerated St. John's Wort (*Hypericum Perforatum* L.cv. Anthos) plants. Plant cell Rep. 19:698-704

9. Ojha AK, Maiti D, Chandra K, Mondal S, Das sadhan K, Roy D, Ghosh K and Islam SS. Structural assignment of a heteropolysaccharide isolated from the gum of *Cochlospermum religiosum* (Katira gum). *Carbo Rese* **2008;** 343: 1222-1231.

10. Rammurthy N and Savithramma N (2002). *In vitro* regeneration of a medicinal plant *Cassia alata* through axillary bud. *J. Plant Biol*. 29: 215-218.

11. Rammurthy N and Savithramma N (2003). Shoot bud regeneration from leaf explants of *Albizzia amara*. *Indian. J. Plant Physiol.* 8: 370-376.

12. Rammurthy N, Savithramma N, Usha R and Swamy PM (1999). Multiple shoot induction and regeneration of Japhara (*Bixa orellana L.*) through axillary bud derived callus cultures. *J. Plant Biol*. 26:231-235.

13. Ranju K, Harsh K. and Shashi VK. *In vitro* multiple shoot production in Sugarcane Genotypes. *J. Plant Biol* **2004;** 31: 65-68.

14. Sasikala A and Savithramma N. Biological synthesis of silver nanoparticles from *Cochlospermum Religiosum* and their antibacterial efficacy. J Pharm Sci Res 2012; 4: 1836-1839.

15. Sasikala A and Savithramma N. Phytochemical divaersity of *Cochlospermum religiosum* (L.) Alsten – A medicinal tree species. *Threats and Concerns to Biodiversity* 2012; 50-59.

16. Sasikala A and Savithramma N. *In vitro* plant regeneration of *Cochlospermum religiosum* through shoot tip culture. Res J Biot 2012; 7: 78-81.

17. Sasikala A, Lingarao M and Savithramma N. Histochemical studies of *Cochlospermum religiosum* (L.) Aston. Weekly Sci Res J 2013; 1: 1-7.

18. Sasikala A, Lingarao M and Savithramma N. Quantification of primary and secondary metabolites from leaves and stembarks of *Cochlospermum religiosum* (L.) Aston. Int Res J Pharm 2013;

19. Savithramma N, Ankanna S, Beena P and Sasikala A (2010). Micro propagation of *Talinum cuneifolium* (Vahl.) Wild. Through petiole culture. *Asian J. Environ. Sci*. 5: 158-163.

20. Savithramma N, Beena P, Sasikala A, Reddy AK and Saradvathi J (2011). Micropropagation of *Talinum cuneifolium* (Vahl.) Willd. through axillary bud culture in plant tissue culture emerging trends Edited by T. Pullaiah. Regency Publications, Delhi Pp. 103-113.

APPENDICES

Table 1: Aseptic Seedling Formation from the Seeds of *Cochlospermem Religiosum* on Full Strength and Half Strength M.S. Medium Containing Different Concentrations of Sucrose

Full Strength/ Half Strength M.S. Medium	
Concentration of Sucrose	**Percentage of Germination**
0%	100%
1%	75%
2%	25%
3%	nil
4%	nil

Table 2: Effect of Different PGR in MS Medium on Shoot Regeneration from Nodal Explants of *Cochlospermum religiosum*

S. No.	Plant Growth Regulators (mg l^{-1})				Percentage of Shoot Regeneration	Mean No. of Shoots / Explant	Mean Length of Shoots in cms
	BAP	KN	IAA	NAA			
1	0.5	-	-	-	NR	-	-
2	1.0	-	-	-	NR	-	-
3	1.5	-	-	-	35	1.4±0.02	2.5±2.01
4	2.0	-	-	-	80	3.9±0.17	4.2±1.18
5	2.5	-	-	-	60	2.7±0.72	4.7±0.03
6	-	0.5	-	-	NR	-	-
7	-	1.0	-	-	NR	-	-
8	-	1.5	-	-	20	1.35±0.17	1.3±0.54
9	-	2.0	-	-	40	2.0±0.65	3.5±0.41
10	-	2.5	-	-	20	1.8±0.28	1.9±0.76
11	1.5	1.5	-	-	35	2.05±0.32	2.8±0.52
12	2.0	2.0	-	-	40	3.62±0.47	3.2±0.13
13	2.5	2.5	-	-	30	1.72±0.61	1.7±0.38
14	2.0	-	0.5	-	10	0.5±0.23	1.0±0.86
15	2.0	-	1.0	-	15	0.8±0.86	0.5±0.73
16	2.0	-	1.5	-	NR	-	-
17	2.0	-	2.0	-	NR	-	-
18	2.0	-	-	0.5	07	0.5±0.07	0.7±0.38
19	2.0	-	-	1.0	05	0.4±0.52	0.3±0.36
20	2.0	-	-	1.5	NR	-	-
21	2.0	-	-	2.0	NR	-	-

Values are means of 12 replicates. Observations after 8 weeks of inoculation. '±' indicates the standard error.

Table 3: Effect of Different Plant Growth Regulators on Callus Induction from Nodes Explants of *Cochlospermum religiosum*

Explant	Plant Growth Regulators in mg l^{-1}				Nature of Response and Morphogenic Ability	
	BAP	NAA	IAA	IBA		
Nodes	1.0	0.5			Greenish yellow calli	+
	1.0	1.0			Light brown calli	+
	1.0		0.5		Light brown calli	+
	1.0		1.0		Light brown calli	+
	1.0			0.5	Darkbrown calli	-
	1.0			1.0	Dark brown compact calli	-

+' represents organogenetic ability of callus, '-' non organogenetic ability of callus,

Table 4: Effect of PGR on Multiple Shoot Regeneration from the Callus Derived from Nodal Explants of *Cochlospermum religiosum*

Callus Source	Plant Growth Regulators in mg l^{-1}		Percentage of Shoot Regeneration	Mean No. of Shoots / Explant	Mean Length of Shoot in cms
	BAP	KN			
Nodal segments	2.0	-	50	3.0±1.03	3.3±0.83
	2.5	-	67	4.0±1.76	3.24±1.16
	-	2.0	42	3.3±0.22	2.82±1.32
	-	2.5	45	2.3±0.13	2.02±2.36

Values are means of 12 replicates. Observations after 8 weeks of inoculation.
'±' indicates the standard error.

Table 5: Effect of Different PGR in Half Strength MS Medium on Root Induction of *in vitro* Raised Shoots of *Cochlospermum religiosum*

S. No	Plant Growth Regulators (mg l^{-1})			Percentage of Root Regeneration	Mean No. of Roots/ Explant	Mean Length of Roots (cms)
	IAA	IBA	BAP			
1	0.5	-	-	25	7.04±1.32	2.8±0.76
2	1.0	-	-	30	9.62±2.13	2.7±1.18
3	1.5	-	-	42	12.09±±1.55	3.64±0.16
4	2.0	-	-	50	13.63±0.05	3.21±0.02
5	2.5	-	-	40	10.76±1.12	3.89±1.17
6	-	0.5	-	30	8.72±1.43	2.87±0.82
7	-	1.0	-	63	12.13±0.34	3.6±1.38
8	-	1.5	-	60	12.01±0.76	3.85±1.72
9	-	2.0	-	45	10.72±0.93	3.54±1.64
10	-	2.5	-	38	10±1.74	3.0±2.18
11	1.0	-	0.2	65	17.4±2.72	3.24±1.48
12	1.0	-	0.3	55	14.3±1.84	4.1±0.06
13	1.0	-	0.4	40	15.12±1.43	3.9±1.73
14	1.0	-	0.5	38	15±1.32	3.0±2.31
15	2.0	-	0.2	39	16±0.12	3.5±1.72
16	2.0	-	0.3	50	16.3±0.05	4.57±0.03
17	2.0	-	0.4	63	15.9±2.01	4.62±0.94
18	2.0	-	0.5	70	19.5±3.57	4.88±1.74

Values are means of 12 replicates. Observations after 8 weeks of inoculation.
'±' indicates the standard error.

Figure 1: Micropropagation of *Cochlospermum religiosum* from Accept Nodal Explants a) Seed Germination; b) *in vitro* Nodal Explants Inoculation on 2 mg l^{-1} BAP; c) Production of Callus on 1.0 mg l^{-1} BAP with 0.5 mg l^{-1} NAA; d) Multiple Shoot Regeneration on 2.5 mg l^{-1} BAP; e) *in vitro* Shoot Rooted on Half Strength MS Medium with 1 mg l^{-1} IAA and 0.2 mg l^{-1} BAP and f) Plantlet during Hardening